Meeting Report

Mechanism-based Toxicology in Cancer Risk Assessment: Implications for Research, Regulation, and Legislation

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The National Toxicology Program (NTP) has broad responsibilities for: 1) expanding the toxicology database on the impact of chemical interactions with biological systems; 2) providing data that strengthen the science base for regulatory decisions; 3) developing and validating alternative test systems; and 4) communicating strategies and findings to the scientific community, regulatory agencies, and the public. To meet these responsibilities, NTP strategies and approaches are evolving along a number of fronts. The overall objective of these initiatives is to more efficiently test chemicals for toxic effects using a broad array of test systems and to generate data that strengthen the scientific foundation on which risk assessments are based. To develop strategies to improve the ability to meet these goals, the NIEHS/NTP sponsored the Workshop on Mechanism-based Toxicology in Cancer Risk Assessment: Implications for Research, Regulation, and Legislation, held 11–13 January 1995 in Research Triangle Park, North Carolina.

Recent legislative and government acts and authorities have called for improvements in risk assessment and prevention strategies. Moreover, the 1992 Advisory Review report of the NTP Board emphasized the need for more mechanism-based studies, and there has been increasing emphasis by NTP scientists over the past few years in incorporating mechanistic considerations in studies. The need to develop approaches for using mechanistic information in toxicological evaluations is emphasized by the fact that of the 70,000 chemicals currently in commerce, adequate toxicological data are available for only 10–20%. Although traditional toxicity tests such as the two-year rodent bioassay have been the basis for most regulatory decisions regarding the safety of environmental chemicals, only a limited number of chemicals can be studied by this approach. Current risk assessment approaches frequently use default assumptions which reflect an inadequate scientific foundation for assessing risk. While good epidemiology studies are desirable, they are relatively insensitive and tend to detect an effect that has already occurred; toxicology, mechanistic, structure activity, and predictive toxicology studies can be part of a prevention strategy. Thus, the crux of the problem is at least twofold: how can adequate toxicology information be developed on more chemicals of public health importance while at the same time providing data for strengthening the scientific foundation and reducing the uncertainty in deriving human risk estimates. Increased knowledge of mechanisms can help in several ways.

An evolving NTP program in mechanism-based toxicology could draw on the tools of molecular biology, which can characterize interactions of chemicals with critical target genes, to provide viable approaches for the development of more accurate and inexpensive methods to perform not only the first step in risk assessment (i.e., hazard identification), but also contribute to determining quantitative dose–response relationships and establishing biomarkers for estimating human exposure.

The NTP is attempting to integrate mechanistic data, human data, toxicity test data, and biomathematics to develop methods for strengthening the scientific foundation on which risk assessments are based. Resources from throughout the institute and participating agencies are being drawn together to accomplish these new initiatives. This process is just beginning and will result in a gradual reorientation of NTP programs.

Workshop Goals and Structure

The overall goals of the workshop were to 1) assess the scientific foundation for using mechanism-based toxicology to address critical issues in risk assessment, 2) identify and propose solutions to the regulatory problems that may emerge by the use of mechanistic toxicology in conducting risk assessments, and 3) determine the applicability of mechanism-based toxicology in conducting risk assessment to current legislative issues. The workshop included the needs, views, and issues of federal and state regulatory agencies, industry, labor, environmental organizations, the legislative branch, the broader scientific community, and the public.

The structure of the workshop included a plenary session in which leading figures in the scientific and regulatory communities defined the issues. (Plenary speakers are listed in the box insert.)

The workshop centered around five different work groups, reflecting five uses of mechanistic toxicology: screening chemicals and setting priorities for carcinogen testing; hazard identification; determining dose–response relationships; species extrapolation; and determining distributions of risk based on genetic predisposition, age, gender, nutrition, and other factors. Work groups were asked to develop recommendations and to identify areas of consensus, disagreement, and knowledge gaps and how to address those gaps.

Each work group consisted of two cochairs, a rapporteur, and approximately 10–12 other invited participants. In addition, there were 20–30 observers in each group who participated in the meeting.

Overall Recommendations

There were several recommendations that were developed during the course of the workshop. There were extensive discussions on these recommendations at the final plenary session and in the public comment session which followed. Workshop recommendations will help the NTP set research priorities and will also help make more effective links between science and policy. If implemented, the recommendations will strengthen the scientific foundation on which risk assessments are based and will address some of the concerns that have spawned recent risk assessment legislation. The recommendations clearly state that the NTP should become more active in developing approaches to generate scientific data to reduce uncertainty in risk assessments. A number of overall recommendations were consistently expressed during the plenary sessions, public comments, and work group deliberations:

- Mechanistic and risk assessment considerations should be incorporated into experimental design and data analysis. Such considerations also should be used in the selection of chemicals to be evaluated for carcinogenicity by the NTP.

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The complete work group proceedings are available from the NTP Liaison Office, NIEHS, PO Box 12233, Research Triangle Park, NC 27709 (FAX: 919-541-0295). This report will help guide the NTP's research priorities in the coming years and will also help in solving the problems encountered by regulatory agencies in using mechanistic information in risk assessment.

• Uncertainty factors in risk assessment should be a critical feature in experimental design, especially interindividual variation, dose–response relationships, and species extrapolation.

• Less expensive, faster, and more accurate methods for setting priorities and providing toxicological evaluations need to be developed and validated. These methods would range from *in vitro* methods to modified *in vivo* approaches, including transgenics.

• Experimental models for evaluating agents or classes of agents should be selected by considering the knowledge gaps that need to be filled for that agent or class of agents.

• The NTP should continue, for the foreseeable future, long–term bioassays, which provide valuable information. NTP and regulatory reliance on long-term bioassays should only be diminished when alternative methods are appropriately validated.

• The acquisition of molecular and biochemical data from exposed populations is critical for risk assessment. The NTP should improve the linkage between experimental toxicology and molecular epidemiology as part of the process of providing toxicological evaluations of public health interest.

• The NTP, working closely with stakeholders, should develop guidelines for achieving regulatory acceptance of mechanistic information in risk assessment. These guidelines should be flexible in order to accommodate the wide variety of mechanisms likely involved in chemical carcinogenesis, the recognition that knowledge of mechanisms will never be complete, and the continued evolution of scientific knowledge.

• The NTP should play a more active role in coordinating overall approaches to incorporate mechanistic information in risk assessment, including sponsoring workshops on specific topics.

• Effective communications with all stakeholders including industry, unions, environmental groups, legislators, and scientists will be essential in attempts to improve the credibility of risk assessment.

Individual Work Groups and Recommendations

Screening Chemicals and Setting Priorities for Carcinogen Testing

The focus of this group was on strategies for developing rational approaches for identifying agents that should be further evaluated and for testing classes of chemicals that share common structural or biological properties. Alternative or flexible designs of bioassays that could affect the understanding of the hazards of a series of chemicals were an important aspect of this discussion group. Topics discussed included alternative test systems, types of mechanistic endpoints most useful in predictive toxicology, validation of short-term tests, and evaluation of mixtures. The group also discussed communication strategies that are needed to achieve regulatory acceptance of alternative methods and the need to involve all stakeholders in developing strategies for using mechanistic information in priority setting.

Recommendations. The establishment of a peer-reviewed document is recommended to form the basis for predicting outcomes of rodent bioassays. This document would include: 1) synthesis of all experimental data that indicate that the agent is very likely to prove carcinogenic to rodents, unlikely to be carcinogenic, or gives mixed signals of carcinogenic potential, 2) if likely to be carcinogenic, how the activity could be most readily assessed/ defined (strain/species, etc.), 3) if carcinogenic in a chronic bioassay, do the predictive and mechanistic data indicate a probable commensurate hazard to humans or a rodent-specific effect?

The NTP should develop categories of mechanistic endpoints to evaluate environmental agents with the standard bioassay. In this manner NTP can validate the predictive nature of mechanistic/short-term studies. Until this method is validated and accepted by all concerned parties, there should be no diminution of bioassays.

The NTP should embark on an educational campaign. The purpose of this educational campaign would be to develop scientific consensus, broaden current thinking as to what is necessary to determine carcinogenicity, and deal with the problems posed by legal precedents. The NTP should explore a partnership with International Agency for Research on Cancer, EPA, and other interested entities to accomplish this educational goal.

Carcinogen Hazard Identification

Strategies for identifying or predicting chemicals reasonably anticipated to be carcinogenic to humans in the absence of two-year rodent bioassays were addressed in this work group. The type and extent of data necessary (for example, structure activity, genotoxicity, receptor-mediated effects, and other biological activities) to predict and classify a chemical as potentially hazardous were discussed. The intent is to identify potential carcinogens on the basis of mechanisms or to provide strong evidence predicting a negative response. The extent to which

Workshop Plenary Speakers

Kenneth Olden, director
National Institute of Environmental Health Sciences and the National Toxicology Program

George Lucier, director
Environmental Toxicology Program, National Institute of Environmental Health Sciences

Lynn Goldman, assistant administrator
U.S. Environmental Protection Agency

Bryan Hardin, senior scientist
National Institute for Occupational Safety and Health

Tom Sinks, associate director for science
National Center for Environmental Health, Centers for Disease Control

Mark Brown
Office of Technology Assessment, U.S. Congress

Barry Gold, professional staff member
Subcommittee on Energy and Environment, Committee on Science, U.S. House of Representatives

J. Carl Barrett, scientific director
National Institute of Environmental Health Sciences

Bernard Schwetz, director
National Center for Toxicological Research and Associate Commissioner for Science, U.S. Food and Drug Administration

Raymond Tennant, chief
Laboratory of Environmental Carcinogenesis/Mutagenesis, National Institute of Environmental Health Sciences

this information can be used for setting priorities for further studies and for regulatory actions was a key point in the discussion.

Recommendations. Although the emphasis on long-term bioassays to assess carcinogenic potential in experimental animals should not be diminished, long-term studies should not be conducted when alternative data suggest little need to conduct a bioassay to address the question of hazard identification.

The NTP is evaluating transgenic mice with an activated Ha-*ras* gene and those in which a single copy of the p53 gene has been inactivated. The results of these studies are promising, and further studies, including validation and the creation of mice with other targeted genes, may merit consideration.

Predicting carcinogenic potential is an analytical process that requires significant scientific judgment and should be done on an ad hoc, case-by-case basis. Characterization of an agent as "likely" or "not likely" to be a carcinogen based on mechanistic information should be accompanied by a statement detailing 1) the basis for the classification, 2) the degree of confidence in the prediction (low, medium, or high, for example), 3) the mechanistic relevance of the data from a particular assay or study used in the assessment, 4) the degree of validation of the approaches used, 5) the empirical predictive power of each assay or study used, and 6) the consistency of responses across all of the endpoints evaluated.

The NTP and other groups should be encouraged to continue to pursue the development of alternative, mechanistically based methods for predicting carcinogenicity. As part of the development process, the NTP, possibly together with other national and international institutes, should organize approach (assay)-specific workshops so that promising areas of research can be discussed in depth for their utility, limitations, and needs. Examples of promising approaches discussed that may warrant a workshop are: development and use of transgenic mice (genes include cancer genes, genes involved in metabolism, and bacterial reporter genes such as *lac*), structure–activity relationships, cell transformation (including human cells), and molecular data from exposed human populations (this area is currently underrepresented by the NTP, but may be a rich source of mechanisms on human carcinogenesis).

Determining Dose-Response Relationships for Chemical Effects and Low-Dose Extrapolation

This group discussed the use of mechanistically important endpoints other than tumor formation for determining dose–response relationships and low-dose extrapolation and the impact of this approach to risk assessment. The kinds of data most useful for low dose risk estimates were evaluated in relation to availability of comparable data in humans. Experimental and mathematical approaches to dose-effect relationships and the data necessary to predict tumor responses from mechanistic-based endpoints within the framework of multistage models were addressed. Of particular interest is the development of credible strategies for the NTP to provide data more usable for low-dose risk estimates.

Recommendations. Definition of a mode of action is critical to establishing a mechanistically based dose–response curve. Mode of action is defined as one or more obligatory steps in a carcinogenic process such as interaction of a chemical with a cellular receptor. Mechanism of action implies a more complex description of the myriad primary and secondary effects, interactions, and alterations that occur in chemical carcinogenesis. Establishment of either comparable evidence or plausibility based on data for related compounds for the mode of action in both rodents and humans (or human tissues) is critical for interspecies extrapolation and dose–response modeling.

The NTP should make an effort to develop methods for measuring biochemical, cellular, and molecular endpoints in both rodents and humans to aid in the extrapolation of risks across the two species. Where possible, these measurements should also be obtained in humans concurrent with rodent assays. Thought should be given to the establishment of databases for these endpoints in both humans and animals (especially in untreated cohorts) to aid in assessing interindividual variability.

The NTP should consider routinely incorporating changes in the bioassay design to address unanswered questions that lead to uncertainties in dose–response evaluation, including the collection of mechanistic data from the 90-day subchronic studies. Multitime, multidose studies may be conducted to provide additional information on pathogenesis in relation to dose.

As much data as possible should be routinely collected at the individual animal level, and these data should be available to the scientific community. This would help in evaluating dose response, describing the full range of expected risks, and in identifying molecular or biological endpoints most

Work Group Co-Chairs

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Marilyn Wind, U.S. Consumer Product Safety Commission
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Facilitator, Bernard Schwetz, National Center for Toxicological Research

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Determining Distributions of Risk
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predictive of risk. Endpoints such as cell proliferation, mutations of critical target genes, and altered expression of target genes might be especially useful.

The NTP should develop methods and data to provide a better link than administered dose between the chronic study for cancer and the short-term studies. These studies may require significant extensions of the dose–response range to accommodate mechanistic data.

Support for research on mode of action should be hypothesis-driven, using structure–activity relationships and other predictive measures to maximize the generation of usable data. Regulatory data-gathering schemes should provide incentives for the generation of quantitative data that expedite and improve regulatory decisions.

Guidelines should be developed to create a framework for acceptance of mechanistic approaches that override current default positions for estimating dose–response relationships, although in the absence of mechanistic data, regulatory agencies may be forced to use certain default positions to evaluate risk under tight time constraints. Statistical and mathematical procedures for handling these data and characterizing models should be developed and implemented. Ad hoc, quantitative descriptions of response without concern for statistical considerations should be avoided.

Species Extrapolation

Given that mechanisms of action are never completely understood, how can current advances in mechanisms of action of chemicals be used to predict responses in different species for different classes of chemicals? What chemical classes are currently understood sufficiently to allow this analysis? What additional classes need to be studied? How does one deal with the situation where a chemical exhibits multiple mechanisms? What constitutes a sufficient body of evidence that a tumor is related to a mechanism with little relevance to humans, or how can knowledge of mechanism be used to strengthen the use of animal data for estimating human risks? Can a general research strategy or approach be developed that can direct necessary research to support mechanistic arguments and better link science to risk assessment?

Recommendations. Mechanistic data can be used initially in a qualitative determination of the biological relevance of a rodent bioassay for estimating human risks. This determination may suggest that it is not appropriate to infer a cross-species cancer risk, and risk assessment should not be pursued for this particular finding. However, simply demonstrating that a mechanism found in a rodent strain does not exist in humans is not sufficient; the mechanism must be demonstrated to be involved in producing tumors in rodents.

The NTP should identify bioassays where significant species differences or similarities in response exist to help the scientific community focus efforts to validate proposed biomarkers, further establish cancer mechanisms, and enhance understanding of interspecies extrapolations. In the validation of new mechanism-based methods for toxicology and carcinogenicity studies, the NTP should not limit comparisons of new methods with data from established rodent models, but should also consider existing data relating to recognized human risks.

Compounds with positive results in validated mechanistic studies can be reasonably expected to be potentially carcinogenic. This determination should be made on a weight-of-the-evidence basis, and may support the following decisions: 1) qualitative hazard identification and appropriate interventions to reduce exposures, 2) prioritization of substances for further testing, including the rodent bioassay, 3) classification decisions, in which case classification rules may need to be modified so that appropriate mechanistic information can be used, and 4) quantitative risk assessment (with information on dose response from mechanistic studies and pharmacokinetic data).

Distributions of Risk

Individuals (humans and rodents) vary greatly in their responses to chemically induced toxicity. Given this fact, current risk assessment methods assume that the most susceptible rodent represents the most susceptible human. This assumption could overestimate or underestimate risks to humans. Given the recent advances in identifying the basis of genetic susceptibilities for chemicals in humans and rodents as well as age and gender-dependent differences, how can we design experiments to determine the distribution of risks among different populations? Another topic was the use of mechanistic approaches to more accurately determine exposure so that more accurate assessments of gene/environment interactions can be made.

Recommendations. The NTP should take an active role in coordinating research on gene/environment interactions to improve understanding of the range of risks expected in general or worker populations as a consequence of chemical exposures. When possible, risk assessment models should take into account human biomarker validation and individual misclassification issues. False positive and negative results have major social, economic, and emotional consequences for those misclassified individuals. These issues are critical topics for future workshops by NTP.

The acquisition and use of data that will be acquired over the next decade will require building trust among community, scientific, regulatory, industrial, occupational, and at-risk constituencies. At the present time, this trust is generally lacking; as researchers move from population-based to individual-based risk assessment strategies, outreach to build this trust becomes more important. The NTP should take an active role in bringing together representatives from these various constituencies to discuss these issues.

The NTP should take a leadership role in coordinating and developing approaches to maximize the use of biomarkers in animal models and in human samples to better understand distribution of risk. Research at all levels relevant to the distribution of risk, including genetic predisposition, age, gender, and nutrition, should be considered.

Appendix

Work Group Members

Screening Chemicals and Setting Priorities for Carcinogen Testing

John Walker, U.S. Environmental Protection Agency
Sidney Green, U.S. Food and Drug Administration
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Carcinogen Hazard Identification

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